Liver Function Tests

(not too broad of a topic…)

Liver Function Tests

- A misnomer
  - elevated aminotransferases/alkaline phosphatase are only markers of **liver injury**, not liver dysfunction
  - Albumin/Bili/PT can be affected by extrahepatic factors
    - nutritional state
    - hemolysis
    - antibiotic use
- Poor sensitivity and specificity for liver disease

“True liver tests”

- Galactose clearance
- Caffeine Clearance
- Aminopyrine Breath Test
- Lidocaine Metabolite Formation
- Indocyanine Green
- Sulfobromophthalein Sodium

- “Abnormal test results occur in as many as one-third of patients screened, but the incidence of clinically significant unsuspected liver disease is approximately 1%.”

History

- Systemic symptoms
- Family Hx
  - Hemochromatosis, Wilson's Disease, alpha$_1$ antitrypsin deficiency
  - Gilbert's syndrome, Dubin-Johnson Syndrome, Rotor's syndrome
- Sexual History
- Tattoos
- Illicit drug use
- Travel history

History

- Occupational exposures
  - Chemicals (vinyl choloride, dimethylformamide, 2-Nitropropane, Trichloroethylene)
- Other co-morbid illnesses
  - Autoimmune diseases, IBD, Diabetes Mellitus
- Medications
  - Prescription
  - OTC
  - Herbals, Vitamins

- **Hepatocellular injury (serum aminotransferase elevations)**
  - Acetaminophen
  - Alpha-methyldopa
  - Amiodarone
  - Dantrolene
  - Diclofenac
  - Disulfiram
  - Fluconazole
  - Glyburide
  - Heparin
  - Isoniazid
  - Ketoconazole
  - Labetalol
  - Lovastatin
  - Nitrofurantoin
  - Propylthiouracil
  - Trazodone
- **Cholestatic injury (serum ALP and bilirubin elevations)**
  - Androgenic anabolic steroids
  - Captopril
  - Chlorpropamide
  - Erythromycin
  - Estrogenic steroids
  - Floxuridine
  - Gold salts
  - Methimazole
  - Phenothiazines
  - Tolazamide
  - Tolbutamide
  - Trimethoprim-sulfamethoxazole
- **Mixed hepatocellular-cholestatic injury**
  - Flutamide
  - Phenylbutazone
  - Phenytoin
  - Sulfonamides
  - Valproic acid

ETOH

Three categories

- Markers of Liver Injury/Necrosis
- Markers of Cholestatic Liver Disease
- Markers of Liver Function

Aminotransferases

- AST - aspartate aminotransferase
  - Serum Glutamic-oxaloacetic transaminase (SGOT)
- ALT - alanine aminotransferase
  - Serum glutamic-pyruvic transaminase (SGPT)
- Catalyze the transfer of the ά-amino groups of aspartate and alanine acid, respectively, to the ά-keto group of ketoglutaric acid.
- Both normally present in serum at low levels (<30 to 40 U/L)
- Both enzymes released into the blood in increasing amounts with liver damage.

Aminotransferases

- **AST (SGOT) (cytosol and mitochondria)**
  - **Liver**
  - **Cardiac Muscle**
  - **Skeletal Muscle**
  - **Kidneys**
  - **Brain**
  - **Pancreas**
  - **Lungs**
  - **Leukocytes**
  - **Erythrocytes**
- **ALT(SGPT) (cytosol)**
  - **Liver**

Isolated elevated AST

- If ALT normal, then reflective of cardiac or muscle disease.
- Marco-AST
  - Rare
  - AST complexed with an immunoglobulin and is not cleared from the blood
  - Does not indicate serious liver disease
- Drugs
  - Acetominophen, NSAIDs, ACE-I, Niacin, INH, Sulfa, Erythromycin, Fluconazole

Isolated elevated ALT

- 99/19,877 (0.5%) Air Force trainee volunteers had elevated ALT levels
- Cause for elevation found only in 12
  - Hepatitis B - 4
  - Hepatitis C - 4
  - Autoimmune Hepatitis - 2
  - Cholelithiasis - 1
  - Acute appendicitis - 1

Kundrotas et al, Dig Dis Sci 1993;38:2145-50

Aminotransferases

- Poor correlation between the extent of liver cell necrosis and elevation of serum aminotransferases.
- Absolute elevation is poor predictor of outcome of acute hepatocellular disorders
- If ALT <300, then an AST/ALT ratio is usually **greater than two** in ETOH liver disease
  - low serum activity of ALT in alcoholics
  - pyridoxal 5'-phosphate deficiency
  - ALT synthesis requires pyridoxal phosphate more than AST synthesis
  - May not apply in setting of cirrhosis

Diel et al. *Gastroenterology* 1984;86:632

Lactate Dehydrogenase

- Cytosolic enzyme found throughout the body
- LDH-5 isoenzyme corresponds to the liver
- Poor sensitivity compared to aminotransferases
- Poor specificity - even with isoenzyme analysis used
- Acute Hepatic injury
  - ALT:LDH ratio < 1.5, more likely to be ischemic hepatitis as compared to acute viral hepatitis

Cassidy et al, J Clin Gastro 19:118, 1994

Alkaline Phosphatase

- Catalyze the hydrolysis of a large number of organic phosphate esters, optimally at an alkaline pH.
  - Precise function of these enzymes unknown
- Liver - synthesized in the bile duct epithelial cells
- Bone - osteoblastic activity
- Kidneys
- Intestine
  - Blood type O or B
  - Familial
- Placenta- levels may double late in pregnancy

Elevated ALP

- Fractionate ALP
- Check GGT - if elevated most likely of hepatic origin and cholestasis at some level
  - Intrahepatic (localized or diffuse liver involvement)
    - PBC, erythromycin, estrogens, methyltestosterone, HCC
  - Extrahepatic (gallstones or tumors)
  - Granulomatous/Infiltrative Disease
    - Sarcoidosis, Fungal infections, TB, Lymphoma
    - Suggestive when ALP disproportionately elevated compared to bilirubin

ALP

- Other non hepatic causes for elevation include:
  - Hyperthyroidism
  - CHF
  - Hypernephroma
  - Lymphoma
  - Children - up to 3x normal
- Low ALP in:
  - Hypothyroidism
  - Wilson’s disease
  - Hemolysis

GGT

- Catalyzes the transfer of the γ-glutamyl group from γ-glutamyl peptides (glutathione) to other peptides and L-amino acids.
- Elevated in liver, biliary, or pancreatic disease.
- Very sensitive for detecting hepatobiliary disease, but poor specificity
- Used primarily to confirm hepatic origin of elevated ALP

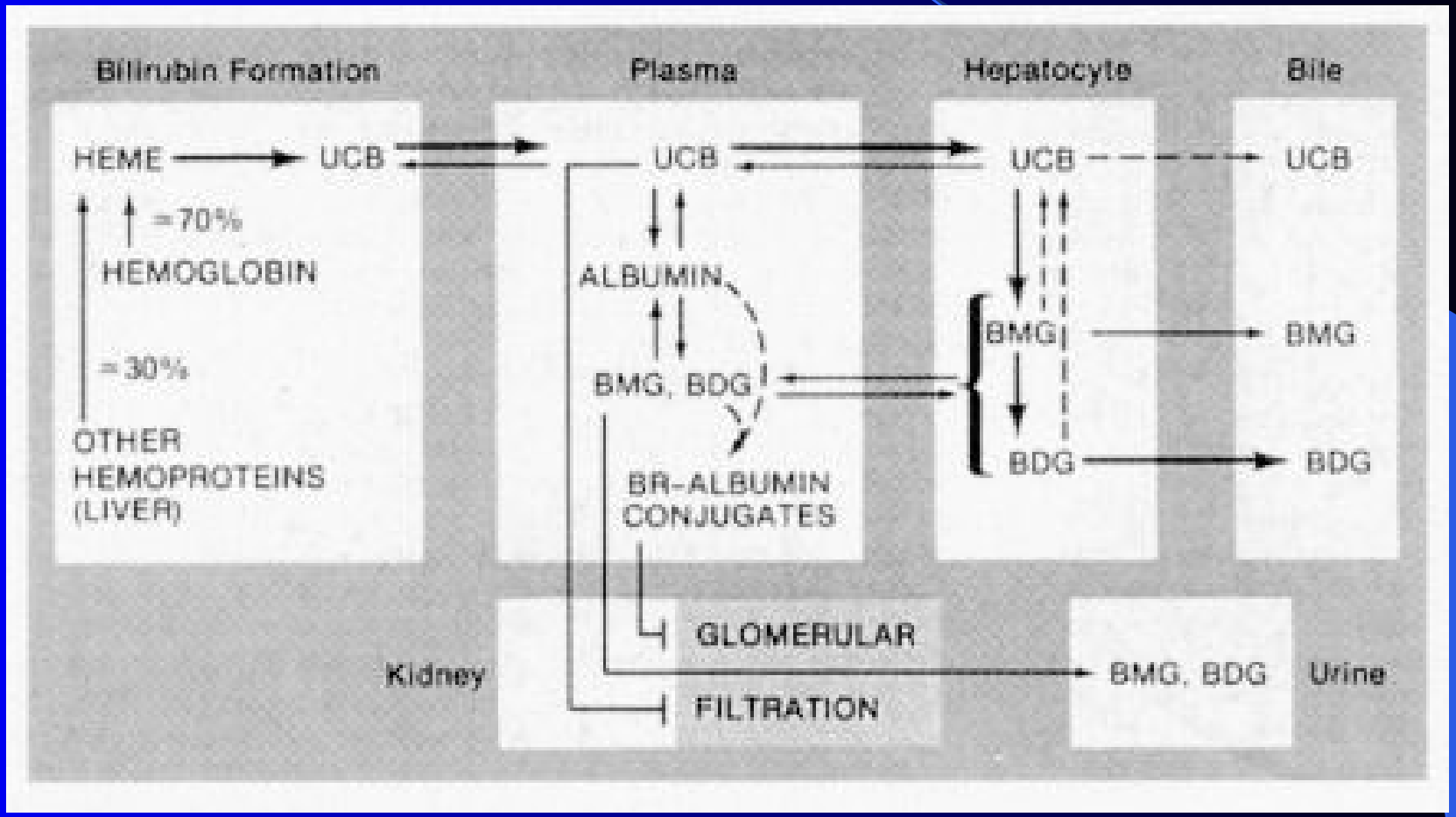
Elevated GGT?

- Pancreatic Disease
- MI
- Renal Failure
- COPD
- DM
- ETOHism
- Drugs - anticonvulsants (phenytoin, barbiturates)

Bilirubin

- Hemoglobin degradation (70-80%)
  - Senescent RBCs (major component)
  - Premature destructions of new RBCs in marrow (minor component)
- Breakdown of nonhemoglobin hemoproteins in liver (20-30%)
- Jaundice usually not seen until bilirubin exceeds 3mg/dL

Bilirubin metabolism



Van den Bergh method

- Bilirubin reacts with diazotized sulfanilic acid
  - **Direct** is fraction that reacts in one minute w/out ETOH
  - **Total** is the amount that reacts in 30 min with ETOH
  - **Indirect** is the difference of direct and total.
- Direct approximates conjugated bilirubin
- Indirect approximates unconjugated bilirubin

Unconjugated Hyperbilirubinemia

- >80% of total bilirubin is indirect
- Liver function is otherwise normal
- **Increased bilirubin production**
  - hemolysis - T.B. seldom > 5 mg/dL
  - ineffective erythropoeisis
  - blood transfusion
  - resorption of hematomas

Unconjugated Hyperbilirubinemia

- Decreased hepatocellular uptake
  - drugs (e.g., rifampin)
  - Gilbert's syndrome?
- Decreased conjugation
  - Gilbert's syndrome
  - Crigler-Najjar syndrome
  - Physiologic jaundice of the newborn

Conjugated Hyperbilirubinemia

- Hepatocellular dysfunction
- Biliary obstruction
- + Urobilinogen
  - unconjugated bilirubin is tightly bound to albumin and not excreted renally
  - marker of hepatobiliary disease

Albumin

- Synthesized exclusively by the liver
- 20 day half life - levels usually preserved acutely
- Synthesis regulated by nutritional states, osmotic pressure, systemic inflammation, and hormones
- Hypoalbuminemia most common in patients with chronic liver disorders (ie cirrhosis) due to decreased synthesis
- Exception: ascites
  - synthesis may be normal or increased, but low serum levels due to increased volume of distribution
- Not specific for liver disease

Prothrombin Time

- Factor 1 - fibrinogen
- Factor II- prothrombin
- Factor V - proaccelerin; labile factor
- Factor VII - stable factor
- Factor IX - Christmas factor
- Factor X - Stuart Prower factor
- Factor XII and XIII - prekallikrein and high molecular weight kinogen

Prothrombin Time

- Parenchymal liver disease
  - Poor utilization of vitamin K
- Hypovitaminosis K
  - Prolonged obstructive Jaundice
  - Steatorrhea
  - Dietary Deficiency
  - Antibiotics (alter gut flora)
- Differentiate by giving IV Vitamin K
  - normalization or 30% improvement within 24 hrs surmises good parenchymal function

Prothrombin Time

- Not a sensitive index of liver disease
  - may be normal or slightly prolonged in severe cirrhosis
- Far more sensitive index of liver synthetic function than albumin
- High prognostic value in acute hepatocellular disease
  - > 5-6 sec above control may be a sign of fulminant hepatic necrosis (ie acute viral hepatitis)
  - ETOH hepatitis
- Higher M/M with diagnostic/surgical procedures

Platelets

- Thrombocytopenia seen in liver dz is thought to be due to congestive splenomegaly
  - Mechanism is platelet sequestration
  - Correlation shown between spleen size and thrombocytopenia
- Platelet count rarely less than 50K
- Bleeding associated with it uncommon
  - Exceptions:trauma, associated platelet function defect
- Congestive splenomegaly does not induce a significant hemostatic defect
- No indication for splenectomy

Platelets

- 198 patients with chronic liver disease
  - 81 liver cirrhosis
  - 68 chronic active hepatitis
  - 49 chronic persistent hepatitis
- Platelet associated IgG (PA – IgG)
  - Measured by ELISA method
  - Inverse correlation of thrombocytopenia with PA-IgG levels

Kajiwara E - *Am J Gastroenterol* - 1995 Jun; 90(6): 962-6

Immunoglobulins

- Elevated in chronic liver disease
  - Antibodies against antigens of normal colonic flora
  - Not taken up and degraded by hepatic RES system
  - Reach extra hepatic lymphoid tissue eliciting inflammatory response
- Persistently hypergammaglobulinemia suggestive of chronic active hepatitis
- Marked increase suggestive of autoimmune chronic hepatitis

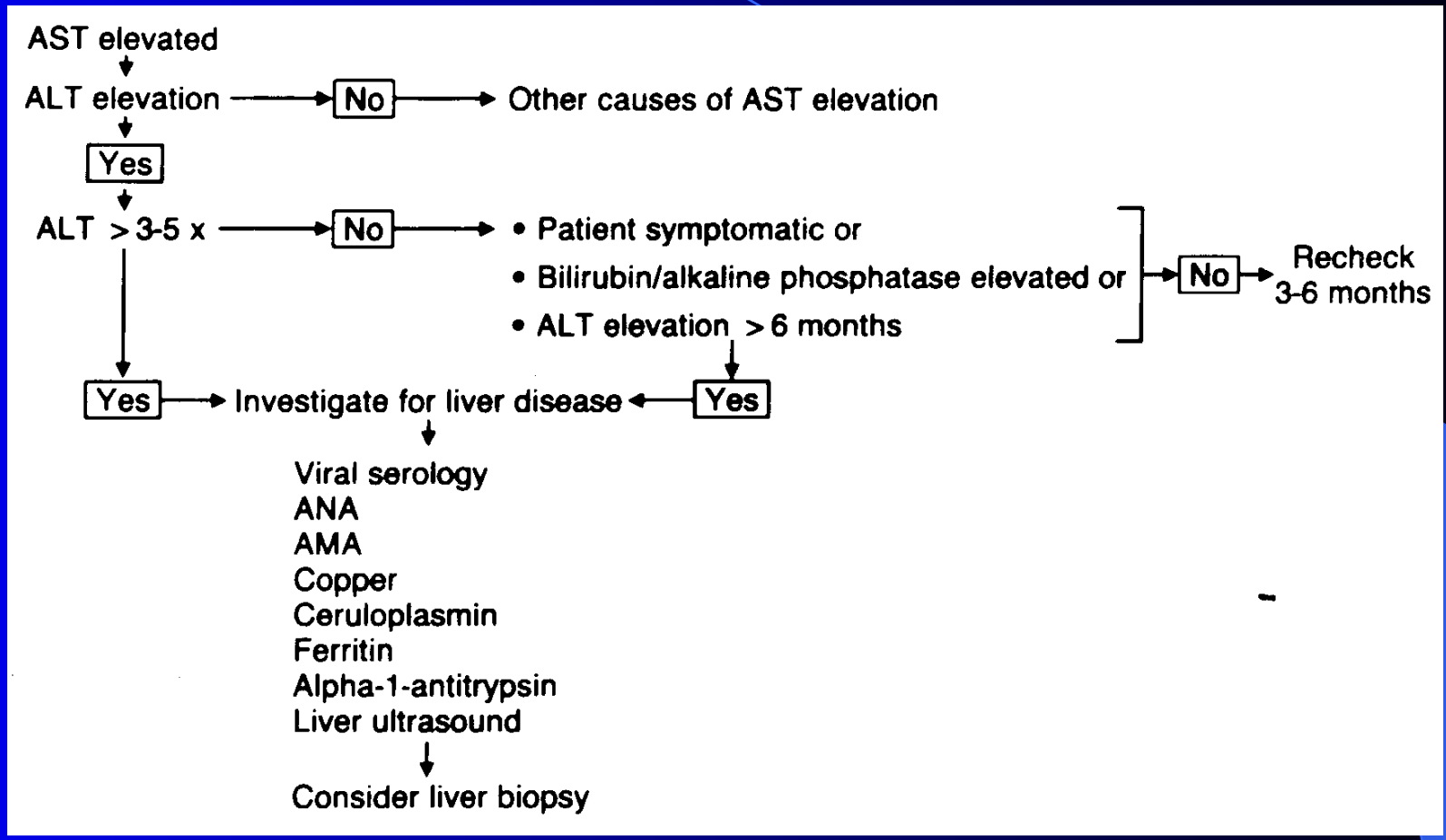
Immunoglobulins

- Most types of cirrhosis
  - Diffuse elevations IgG and IgM
- Primary Biliary Cirrhosis
  - Increase in IgM
- ETOH Cirrhosis
  - Increase IgA
- Useful in monitoring immunosuppresive therapy in pts with autoimmune chronic hepatitis
- Not specific to liver disease

Evaluation of Liver Abnormalities

Hepatocellular Necrosis (mild to moderate) (ALT/AST <250, ALP <200)

- **Common causes**
  - NASH
  - ETOH
  - Hepatitis B
  - Hepatitis C
  - Drugs
  - Hemochromatosis
- **Less common**
  - Wilson's Dz
  - Autoimmune
  - Biliary Tract dz
  - Malignancy
  - α-1 AT deficiency
  - Systemic Illnesses

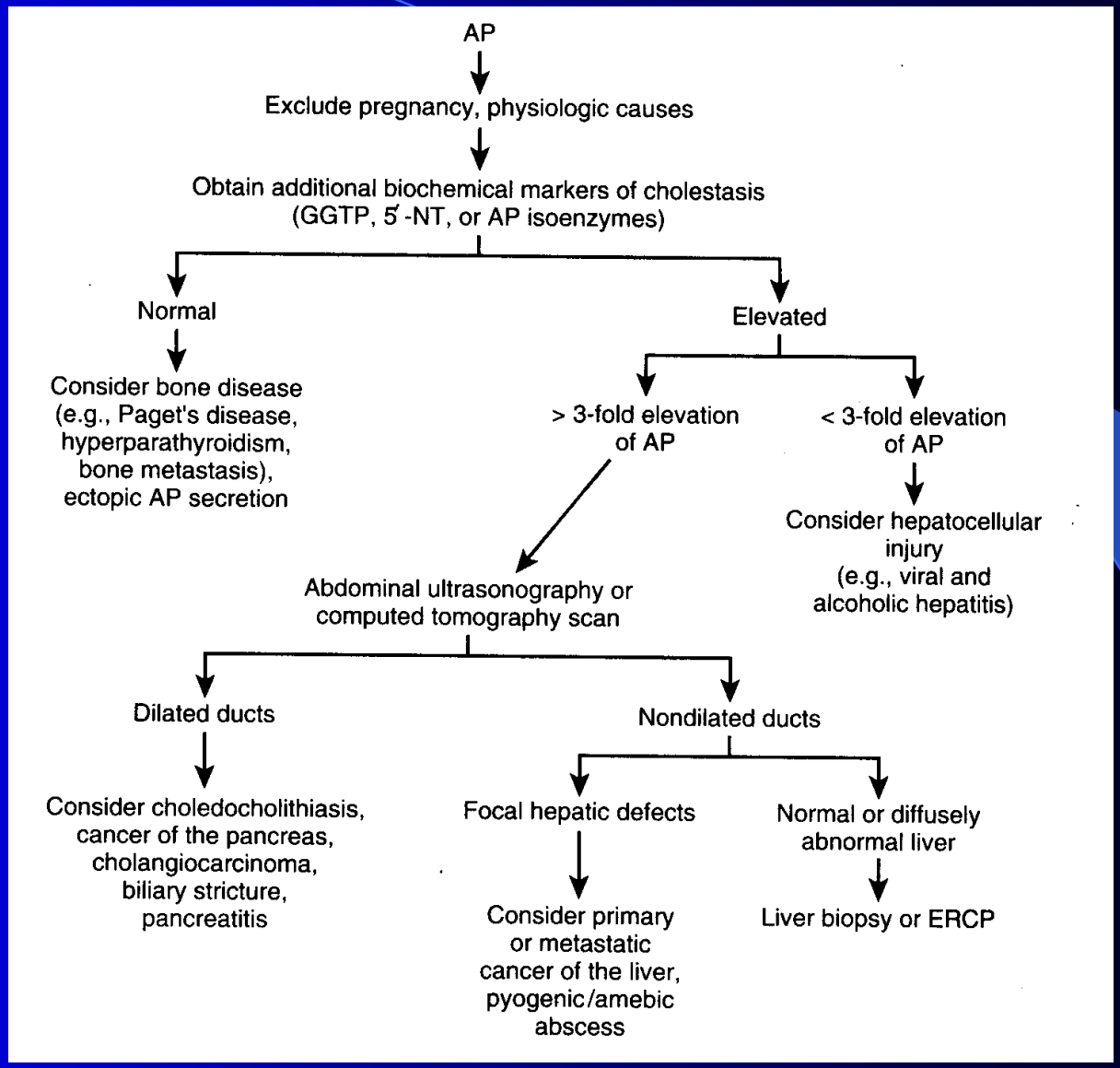

AST elevated
ALT elevation
No
Other causes of AST elevation
Yes
ALT > 3-5 x
No
• Patient symptomatic or
• Bilirubin/alkaline phosphatase elevated or
• ALT elevation > 6 months
No
Recheck
3-6 months
Yes
Investigate for liver disease
Yes
Viral serology
ANA
AMA
Copper
Ceruloplasmin
Ferritin
Alpha-1-antitrypsin
Liver ultrasound
Consider liver biopsy

Hepatocellular Necrosis (moderate to severe) (ALT/AST >250, ALP <200)

- Common
  - Hepatitis A
  - Hepatitis B
  - Hepatitis C
  - Drugs
  - Autoimmune
  - Acute biliary obstruction
- Less common
  - Ischemia
  - NASH
  - CMV
  - Mononucleosis
  - Wilson’s Disease
  - α-1 AT deficiency

Cholestasis
ALP >250

- **Common**
  - Biliary Obstruction
  - Drugs
  - Granulomatous Hepatitis
  - PBC
  - PSC
- **Less Common**
  - Hyperthyroidism
  - Syphilis
  - TPN
  - Metastatic liver disease
  - Amyloid
  - Pregnancy
  - HIV disease
  - Lymphoma
  - Post-op

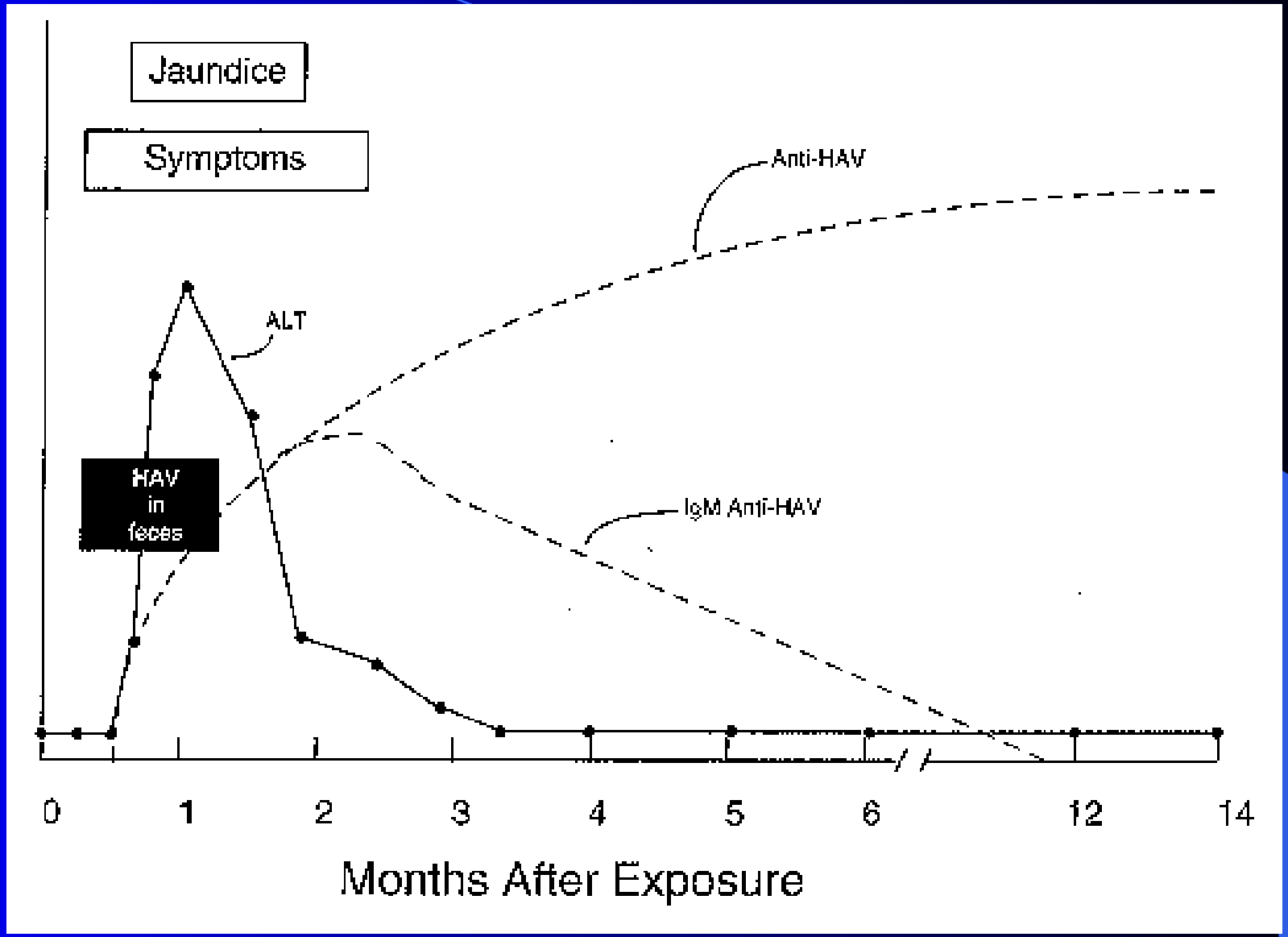

AP
Exclude pregnancy, physiologic causes
Obtain additional biochemical markers of cholestasis (GGTP, 5′-NT, or AP isoenzymes)
Normal
Consider bone disease (e.g., Paget's disease, hyperparathyroidism, bone metastasis), ectopic AP secretion
Elevated
> 3-fold elevation of AP
< 3-fold elevation of AP
Consider hepatocellular injury (e.g., viral and alcoholic hepatitis)
Abdominal ultrasonography or computed tomography scan
Dilated ducts
Consider choledocholithiasis, cancer of the pancreas, cholangiocarcinoma, biliary stricture, pancreatitis
Nondilated ducts
Focal hepatic defects
Consider primary or metastatic cancer of the liver, pyogenic/amebic abscess
Normal or diffusely abnormal liver
Liver biopsy or ERCP

ETOH

- AST:ALT ratio >2
  - usually AST less than 300-500 IU/L
  - If transaminases are higher, must consider concominant drug toxicity (ie acetominophen)
- GGT -
  - If 2x normal with appropriate transaminase ratio, suggestive of ETOH use
- Elevated MCV

Hepatitis A

- 2- 4 week incubation period
- Aminotransferases show an abrupt rise to a peak within 24-48 hrs of first detected abnormality
- Anti-HAV IgM
  - Diagnosis based on positivity
  - Positive from onset of symptoms
  - May remain positive for approximately 4 months
- Anti-HAV IgG
  - Positive from onset of disease
  - Marker of previous infection


Jaundice
Symptoms
Anti-HAV
ALT
HAV in feces
IgM Anti-HAV
0
1
2
3
4
5
6
12
14
Months After Exposure

Hepatitis B

- HBsAg
  - serologic hallmark of HBV infection
  - appears in serum 1-10 weeks after acute exposure
  - appears 2-6 weeks before hepatitis symptoms
  - persistence greater than 6 months implies chronicity
- Anti-HBs
  - Marks recovery from hepatitis B
  - Often not detectable until after “window period”

Hepatitis B

- Anti-HBc (IgM)
  - First antibody to be detected (within one month after appearance of HBsAg)
  - **Sole marker of HBV infection during window period**
  - Usually an indicator of **acute** infection
  - May remain detectable up to two years after acute infection
  - Low-titer IgM may persist in chronic HBV infection

Hepatitis B

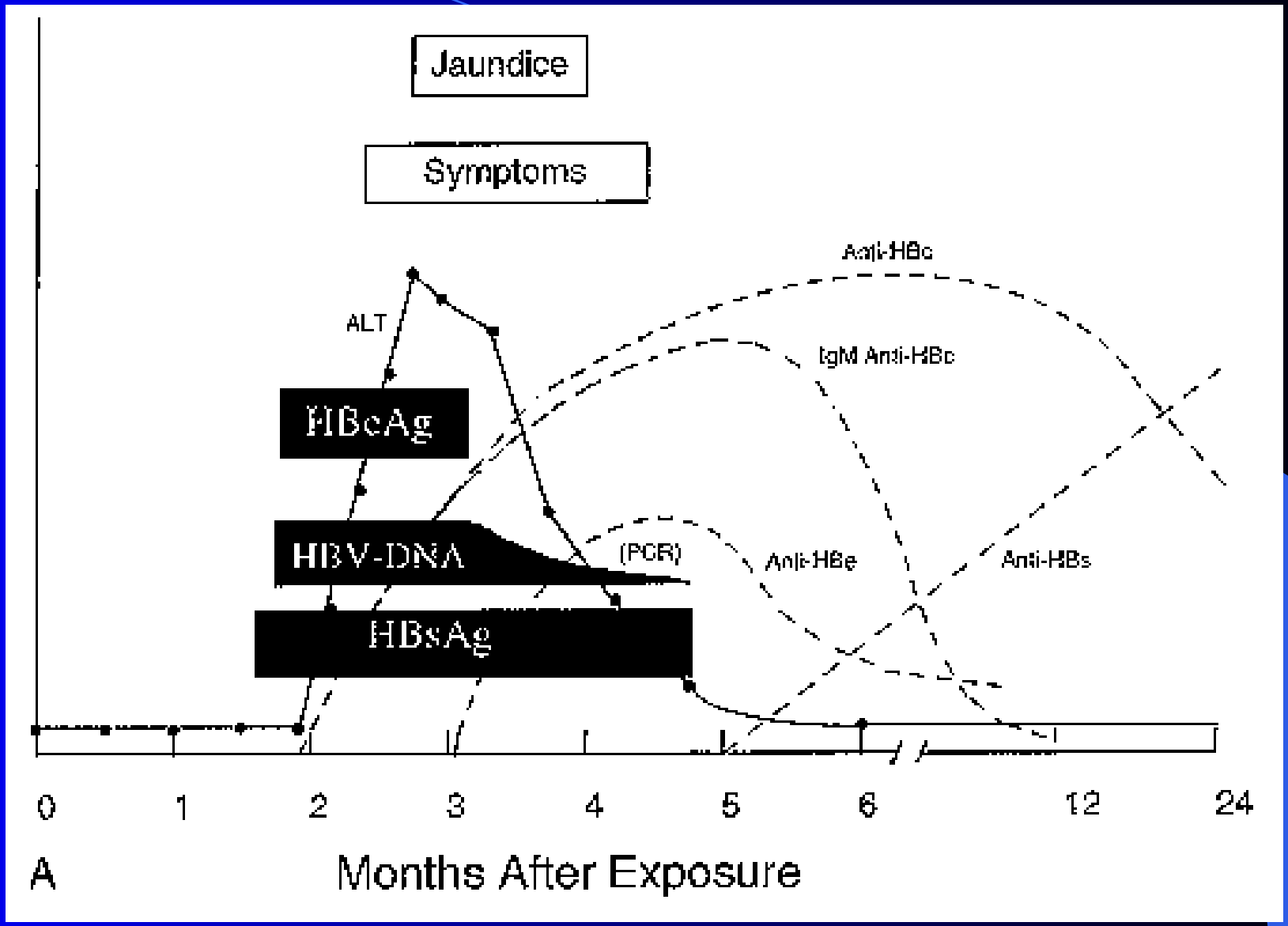
- Anti-HBc (IgG)
  - persists along with anti- HBs in patients who recover from acute infection and those who progress to chronic infection
  - Isolated presence with absence of HBsAg and anti-HBs
    - During window period (although predominantly IgM)
    - Many years after recovery from acute infection when anti-HBs has fallen to undetectable levels
    - Many years of chronic infection when HBsAg titer has fallen to undetectable levels
    - Clinical significance is unclear

Hepatitis B

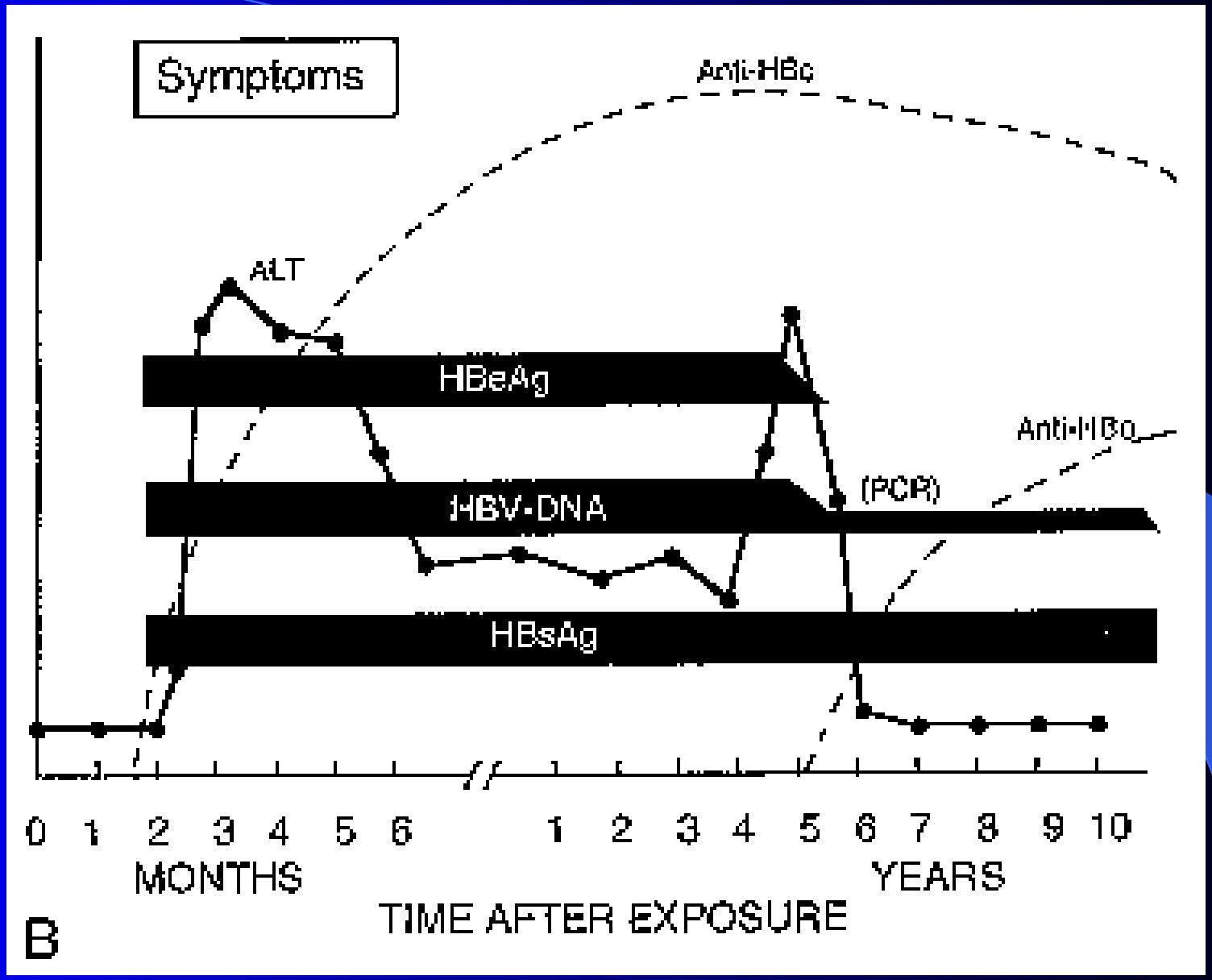
- HBeAg
  - Marker of HBV replication and infectivity
  - Appears shortly after appearance of HBsAg
  - Higher rate of transmission when positive
  - Most have detectable HBV DNA
- Anti-HBe
  - May persist for years after resolution of acute episode
  - Seroconversion usually associated with disappearance of HBV DNA and remission
  - Small proportion of patients still have active disease

Hepatitis B

- HBV DNA
  - Can be detected 1 week after appearance of HBsAg
    - Hybridization assays
  - PCR can detect it up to 2-3 weeks before HBsAg
  - Major role is in chronic infection to assess HBV replication and possibility of antiviral therapy
  - High DNA levels are less likely to respond to interferon therapy
  - May be checked in pts with isolated anti-HBc IgG to rule out low level chronic HBV infection



Acute HBV infection with resolution



Acute HBV infection converting to chronicity

Hepatitis C

- Enzyme immunoassay (screening)
  - EIA 1 - 80% sensitivity, but a high false positive rate (50 to 70%)
  - EIA 2 - 95% sensitivity, 40-50% false positive rate
  - EIA 3 - test of choice to screen blood products; similar sensitivity and specificity of EIA 2
- HCV RNA
  - PCR or branched DNA techniques
  - Confirming diagnosis and assessing response to IFN tx
  - Lack of standardization and lab to lab variability

Hepatitis C

- Aminotransferases
  - 90 patients who were hepatitis C positive
  - Aminotransferase levels and liver histology
  - Aminotransferase levels were not correlated with histological findings (ie inflammation and fibrosis)
  - > 10x elevations (ALT>350) is suggestive of piecemeal necrosis

Haber et al., Am J Gastro, 1995; 90:1250-1257

Autoimmune Hepatitis

- Most frequently women
  - ages 10-30 or late middle age
  - May mimic acute viral hepatitis in presentation
  - Numerous extrahepatic manifestations
  - Types 1,2,3
- Moderately elevated transaminases
- Hypergammaglobulinemia
  - more than 80% of patients
  - SPEP - more than 2x normal of polyclonal IgG suggestive of diagnosis

Autoimmune Hepatitis

- ANA
  - >1:40 titer for significance
  - 28% sensitivity
- SMA
  - Directed against F-actin
  - >1:80 titer
  - May be only marker of autoimmune hepatitis at high titer
  - 40% sensitivity
  - May have low + titers in chronic viral hepatitis, but lack F-actin specificity

Czaja AJ. Semin Liver Dis 1984;4:1-12

Autoimmune Hepatitis

- Liver-kidney microsomal antibodies (LKM)
  - Target antigen is cytochrome P450 2D6
  - Predominantly marker of autoimmune hepatitis type 2
  - Rarely positive in patients in US, Australia, Japan
- Antibodies to cytosolic antigens (SLA, LP)
  - May be only markers in when ANA, SMA, LKM neg.
- Autoantibodies to hepatocellular membrane antigens (ASPGR)
  - May be + when all other tests negative and still suspect diagnosis
- Anti-mitochondrial antibodies (AMA)
  - Should be negative

Alpha 1 Antitrypsin Deficiency

- Homozygous PIZZ ά-1 AT
  - Scandinavian and Northern European descent
  - 1 in 1600 to 1800 births
  - Neonatal liver disease
- Sveger et al, NEJM 1976
  - Screened 200,00 newborns - 127 homozygotes
  - 14 had jaundice
  - At age 18, 85% had normal transaminases with no evidence of liver dysfunction
  - Variable penetrance
- Unclear if heterozygotes are at risk for liver injury

Alpha 1 Antitrypsin Deficiency

- Serum electrophoresis
  - absent alpha 1 globulin peak
- Serum Alpha 1 AT phenotype determination
  - Definitive diagnosis
  - isoelectric focusing or agarose electrophoresis
- Serum Alpha 1 AT levels
  - Often misleading
  - May increase during inflammatory response, thus giving false negative results
- Liver Biopsy
  - Distinctive (not diagnositic) finding of PAS-+, diastase resistant globules in periportal hepatocytes

Hemochromatosis

- HFE gene (cytosine to tyrosine substitution C282Y)
  - Has been identified in 60-100% of patients with hereditary hemochromatosis
  - Burke et al - case series 0.5% to 14% of patients were heterozygotes of HFE gene
- Cases of families with hemochromatosis without HFE defect
- Population screening not recommended, but may be useful in first degree relatives of patients

Hemochromatosis

- Fe - elevated > 170g/100ml
  - need fasting study (meal dependant)
- Ferritin >500 µg/L
  - acute phase reactant
- Transferrin saturation (Fe/TIBC)
  - >45%
  - 98% sensitivity, few false positive results
- Liver biopsy
  - Hepatic iron index of more than 1.9

Wilson's Disease

- Ceruloplasmin
  - Serum glycoprotein that contains six copper atoms
  - Copper incorporation into ceruloplasmin is impaired in Wilson's disease
  - 95% of homozygotes have levels <20mg/dL (rarely are levels >30mg/dL)
  - May also be low in other hypoproteinemic states
  - May be low in 20% of asymptomatic heterozygotes

Wilson's Disease

- Serum free copper (unbound copper)
  - greater than 25 µg in symptomatic pts (nl <10)
- Slit lamp detection of Kayser Fleischer Rings
- 24 hour urinary copper excretion
  - may exceed 100µg/24 h - use metal free container
  - False + with sign. Proteinuria (ceruloplasmin loss)
- Liver biopsy
  - > 250 µg/g copper dry weight in homozygotes (normal <50)
  - Cholestatic diseases (PBC/PSC) may have elevated hepatic copper dry weight

Primary Biliary Cirrhosis

- Cholestatic pattern of injury
  - only 10% of patients jaundiced at diagnosis
- Antimitochondrial antibodies
  - 1:40 in 90% of patients, 1:80 >95%
  - May also be positive in :
    - Autoimmune hepatitis
    - PSC
    - syphillis
    - myocarditis
    - drug induced liver disease

NASH

- Transaminases only mildly elevated
- AST:ALT ratio <1
- Serum ferritin and transferrin saturation may be elevated
  - Does not correlate with high hepatic iron index
  - Hepatic necroinflammatory activity
- Ultrasound - can detect significant steatosis
- CT scan - Liver appears hypodense compared to spleen
- Liver Biopsy

NASH

- Prospective study of 1124 adults with elevated liver enzymes
- 81 of 1124 were marker negative
- Liver biopsy showed
  - 41 had steatosis
  - 26 had steatohepatitis
  - 8 had normal histology
  - 4 had fibrosis
  - 2 had cirrhosis
- In setting of marker negative elevated LFTs, steatosis is most likely histological diagnosis

Daniel et al, Am J Gastro, 1999:94 p3010-3014

Lessons learned

- Look at the entire clinical picture
- Don’t let one lab test make your diagnosis as they are not always 100%
- If all else fails….check the thyroid


the first years